

File 410:Chronolog(R) 1981-1999 Sep/Oct
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File 155:MEDLINE(R) 1966-1999/Dec W3
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Set	Items	Description
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	159	PNEUMOLYSIN
	94542	MUTANT
	198436	MUTAT?
	233789	MODIF?
	64966	ATTENUAT?
S1	32	PNEUMOLYSIN(30N)(MUTANT OR MUTAT? OR MODIF? OR ATTENUAT?)
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	S2	32 RD (unique items)
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2/3,AB/1
DIALOG(R)File 155:MEDLINE(R)
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10065086 99346586
[Selection of virulent mutants of Streptococcus pneumoniae. Utilization of a murine model of septicemia]
Selection de mutants virulents de Streptococcus pneumoniae. Utilisation d'un modele murin de septicemie.
Amory-Rivier C; Rieux V; Azoulay-Dupuis E; Carbon C; Trombe MC
Institut National de la Sante et de la Recherche Medicale, CRI n(o) 9802, Batiment U13, Hopital Bichat-Claude Bernard, Paris, France.
Pathol Biol (Paris) (FRANCE) May 1999, 47 (5) p519-25, ISSN 0369-8114
Journal Code: OSG
Languages: FRENCH Summary Languages: ENGLISH
Document type: JOURNAL ARTICLE English Abstract
Genetic construction of virulence deficient **mutant** is a strategy to analyse virulence genes of Streptococcus pneumoniae and was used to virulence factors as capsule, **pneumolysin**, autolysin and PspA. We perform a model allowing the in vivo positive selection of virulent S. pneumoniae mutants. Mice which are the most susceptible animals to pneumococcal infection, offer the best model for screening virulent S. pneumoniae. Indeed, after intraperitoneal injection of bacterial mix which was composed to a lot of avirulent bacteria (6 log10 CFU per mouse) (V1015 strain, DL50 = 7.05) and few virulent pneumococci (1 to 2 log10 CFU per mouse) (P4241 strain, DL50 < 1), mice cleared all avirulent bacteria but not virulent pneumococci. Thus, mice dead in 3 to 4 days with septicaemia

and positive hemoculture contained only virulent strain. This model was validated by in vivo selection of a virulent mutant (V1042, DL50 = 4.1) which was obtained after transformation of avirulent strain V1015 with the genomic fragment of virulent strain P4241. Our model of screening was the only one allowing detection of virulent *S. pneumoniae* mutants. This new genetic strategy which consisted in gene addition and used mouse as selection agent, could be used to discover new virulence genes required to in vivo bacterial development.

2/3,AB/2

DIALOG(R)File 155:MEDLINE(R)

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10048468 99346155

Pneumolysin, a protein toxin of *Streptococcus pneumoniae*, induces nitric oxide production from macrophages.

Braun JS; Novak R; Gao G; Murray PJ; Shenep JL

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Infect Immun (UNITED STATES) Aug 1999, 67 (8) p3750-6, ISSN 0019-9567
Journal Code: G07

Contract/Grant No.: AI 27913, AI, NIAID; P30 CA 21765, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Nitric oxide (NO) production by inducible NO synthase (iNOS) during inflammation is an essential element of antimicrobial immunity but can also contribute to host-induced tissue damage. Under conditions of bacterial sepsis, large amounts of NO are produced, causing hypotension, a critical pathological feature of septic shock. In sepsis caused by gram-positive organisms, the bacterial factors contributing to host NO production are poorly characterized. We show that a soluble toxin of *Streptococcus pneumoniae*, **pneumolysin** (Pln), is a key component initiating NO production from macrophages. In contrast to wild-type bacteria, a **mutant** of *S. pneumoniae* lacking Pln failed to elicit NO production from murine macrophages. Purified recombinant Pln induced NO production at low concentrations and independently of exogenous gamma interferon (IFN-gamma) priming of RAW 264.7 macrophages. However, IFN-gamma was essential for Pln-induced NO production, since primary macrophages from mice lacking the IFN-gamma receptor or interferon regulatory factor 1, a transcription factor essential for iNOS expression, failed to produce NO when stimulated with Pln. In addition, Pln acts as an agonist of tumor necrosis factor alpha and interleukin 6 production in macrophages. The properties of Pln, previously identified as a pore-forming hemolysin, also include a role as a general inflammatory agonist.

2/3,AB/3

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09997596 99270945

Role of Pneumolysin's complement-activating activity during pneumococcal bacteremia in cirrhotic rats.

Alcantara RB; Preheim LC; Gentry MJ

Veterans Affairs Medical Center, Omaha, Nebraska, USA.

Infect Immun (UNITED STATES) Jun 1999, 67 (6) p2862-6, ISSN 0019-9567
Journal Code: G07

Languages: ENGLISH

Document type: JOURNAL ARTICLE

We investigated the role of **pneumolysin's** complement-activating activity during *Streptococcus pneumoniae* bacteremia in a hypocomplementemic, cirrhotic host. Isogenic **mutant** pneumococcal strains, in which **pneumolysin** was expressed from a plasmid, were used. These strains included H+C+, expressing wild-type **pneumolysin** with both cytolytic and complement-activating activity; **PLY-**, carrying the plasmid without the pneumolysin gene; and, H+C-, expressing pneumolysin with cytolytic activity only. In control rats, intravenous infection with 2.0×10^7 CFU of H+C+ per ml of blood resulted in a decrease in bacteremia of 3.5 log units by 18 h postinfection and 55% mortality. By contrast, cirrhotic rats infected similarly with the H+C+ strain demonstrated a 0.2-log-unit increase in bacteremia by 18 h postinfection and 100% mortality. Both control and cirrhotic rats cleared the **PLY-** strain more effectively from their bloodstreams by 18 h postinfection (6.2 and 5.6 log unit decreases, respectively). Infection with the **PLY-** strain also resulted in low mortality (0 and 14%, respectively) for control and cirrhotic rats. When infected with the H+C- strain (without complement-activating activity), both groups cleared the organism from their bloodstreams nearly as well as they did the **PLY-** strain. Furthermore, the mortality rate for control and cirrhotic rats was identical after infection with the H+C- strain. These studies suggest that pneumolysin production contributes to decreased pneumococcal clearance from the bloodstream and higher mortality in both control and cirrhotic rats. However, pneumolysin's complement-activating activity may uniquely enhance pneumococcal virulence in the hypocomplementemic, cirrhotic host.

2/3,AB/4

DIALOG(R) File 155:MEDLINE(R)

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09861537 99113007

Pneumolysin in pneumococcal adherence and colonization.

Rubins JB; Paddock AH; Charboneau D; Berry AM; Paton JC; Janoff EN

Department of Medicine, Veterans Affairs Medical Center and University of Minnesota School of Medicine, Minneapolis, MN, 55417, USA.

Microb Pathog (ENGLAND) Dec 1998, 25 (6) p337-42, ISSN 0882-4010

Journal Code: MIC

Contract/Grant No.: AI-042240, AI, NIAID; AI-39445, AI, NIAID; HL-57880, HL, NHLBI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The universal and highly conserved production of pneumolysin, the major pneumococcal cytotoxin, among clinical isolates of *Streptococcus pneumoniae* and the previously reported association of **pneumolysin** production with increased pneumococcal adherence to respiratory epithelium in organ cultures suggest that this toxin might be important for nasopharyngeal colonization. We confirmed that **pneumolysin-deficient mutant** pneumococcal strains had decreased adherence to respiratory epithelial cells in vitro compared with their isogenic wild-type strains. However, neither early nor sustained colonization by type 14 *S. pneumoniae* in an established murine model was dependent on bacterial production of pneumolysin. We conclude that pneumolysin production is not a major determinant of successful nasopharyngeal colonization by pneumococci. Copyright 1998 Academic Press

2/3,AB/5

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09859595 99115586

Comparative virulence of *Streptococcus pneumoniae* strains with insertion-duplication, point, and deletion **mutations** in the **pneumolysin** gene.

Berry AM; Ogunniyi AD; Miller DC; Paton JC

Molecular Microbiology Unit, Women's and Children's Hospital, North Adelaide, S.A., 5006, Australia.

Infect Immun (UNITED STATES) Feb 1999, 67 (2) p981-5, ISSN 0019-9567
Journal Code: GO7

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Pneumolysin is a 471-amino-acid toxin produced by *Streptococcus pneumoniae* which has both cytolytic and complement activation properties. We have constructed a derivative of the type 2 *S. pneumoniae* strain D39 in which the portion of the **pneumolysin** gene encoding amino acids 55 to 437 has been deleted in-frame. The virulence of this strain (DeltaPly) was compared with those of wild-type D39, a **pneumolysin** insertion-duplication **mutant** (PLN-A), and a derivative (PdT) carrying a toxin gene with three point **mutations** known to abolish both cytolytic activity and complement activation. PdT was intermediate in virulence between D39 and either PLN-A or DeltaPly in a mouse intraperitoneal challenge model. This provides unequivocal evidence that **pneumolysin** has an additional property that is not abolished by point **mutations** which reduce cytotoxicity and complement activation to virtually undetectable levels.

2/3,AB/6

DIALOG(R) File 155:MEDLINE(R)

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09768643 98451771

Multivalent pneumococcal capsular polysaccharide conjugate vaccines employing genetically detoxified pneumolysin as a carrier protein.

Michon F; Fusco PC; Minetti CA; Laude-Sharp M; Uitz C; Huang CH; D'Ambra AJ; Moore S; Remeta DP; Heron I; Blake MS

North American Vaccine, Inc., Beltsville, Maryland, USA. fmichon@nava.com
Vaccine (ENGLAND) Nov 1998, 16 (18) p1732-41, ISSN 0264-410X

Journal Code: X60

Languages: ENGLISH

Document type: JOURNAL ARTICLE

A genetically detoxified pneumolysin, pneumolysoid (PLD), was investigated as a carrier protein for pneumococcal capsular polysaccharide (CPS). Such a CPS-PLD conjugate might provide additional protection against pneumococcal infections and resultant tissue damage. A single point **mutant** of **pneumolysin** was selected, which lacked measurable haemolytic activity, but exhibited the overall structural and immunological properties of the wild type. PLD conjugates were prepared from CPS serotypes 6B, 14, 19F, and 23F by reductive amination. The structural features of free PLD, as well as the corresponding CPS-PLD, as assessed by circular dichroism spectroscopy, were virtually indistinguishable from the wild type counterpart. Each of the CPS monovalent and tetravalent conjugate formulations were examined for immunogenicity in mice at both 0.5 and 2.0 micrograms CPS per dose. Tetanus toxoid (TT) conjugates were similarly created and used for comparison. The resultant conjugate vaccines elicited high levels of CPS-specific IgG that was opsonophagocytic for all serotypes tested. Opsonophagocytic titres, expressed as reciprocal dilutions resulting in 50% killing using HL-60 cells, ranged from 100 to 30,000, depending on the serotype and formulation. In general, the lower dose and

tetravalent formulations yielded the best responses for all serotypes (i.e., either equivalent or better than the higher dose and monovalent formulations). The PLD conjugates were also generally equivalent to or better in CPS-specific responses than the TT conjugates. In particular, both the PLD conjugate and the tetravalent formulations induced responses for type 23F CPS that were approximately an order of magnitude greater than that of the corresponding TT conjugate and monovalent formulations. In addition, all the PLD conjugates elicited high levels of pneumolysin-specific IgG which were shown to neutralize pneumolysin-induced haemolytic activity in vitro. As a result of these findings, PLD appears to provide an advantageous alternative to conventional carrier proteins for pneumococcal multivalent CPS conjugate vaccines.

2/3,AB/7

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09760597 99033058

The molecular mechanism of pneumolysin, a virulence factor from *Streptococcus pneumoniae*.

Rossjohn J; Gilbert RJ; Crane D; Morgan PJ; Mitchell TJ; Rowe AJ; Andrew PW; Paton JC; Tweten RK; Parker MW

The Ian Potter Foundation Protein Crystallography Laboratory, St. Vincent's Institute of Medical Research, 41 Victoria Parade, Fitzroy, Victoria, 3065, Australia. jamie@brains.medstv.unimelb.edu.au

J Mol Biol (ENGLAND) Nov 27 1998, 284 (2) p449-61, ISSN 0022-2836

Journal Code: J6V

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Pneumolysin, a member of the thiol-activated cytolysin family of toxins, is a virulence factor from the Gram-positive bacterium *Streptococcus pneumoniae*. The toxin forms large oligomeric pores in cholesterol-containing membranes of eukaryotic cells. A plethora of biochemical and mutagenesis data have been published on pneumolysin, since its initial characterization in the 1930s. Here we present an homology model of the monomeric and oligomeric forms of pneumolysin based on the recently determined crystal structure of perfringolysin O and electron microscopy data. A feature of the model is a striking electronegative surface on parts of **pneumolysin** that may reflect its cytosolic location in the bacterial cell. The models provide a molecular basis for understanding the effects of published mutagenesis and biochemical **modifications** on the toxic activity of **pneumolysin**. In addition, spectroscopic data are presented that shed new light on **pneumolysin** activity and have guided us to hypothesise a detailed model of membrane insertion. These data show that the environment of some tryptophan residues changes on insertion and/or pore formation. In particular, spectroscopic analysis of a tryptophan mutant, W433F, suggests it is the residue mainly responsible for the observed effects. Furthermore, there is no change in the secondary structure content when the toxin inserts into membranes. Finally, the basis of the very low activity shown by a pneumolysin molecule from another strain of *S. pneumoniae* may be due to the movements of a key domain-domain interface. The molecular basis of pneumolysin-induced complement activation may be related to the structural similarity of one of the domains of pneumolysin to Fc, rather than the presumed homology of the toxin to C-reactive protein as previously suggested. Copyright 1998 Academic Press.

2/3,AB/8

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09505932 98181051

Amino acid changes affecting the activity of pneumolysin alter the behaviour of pneumococci in pneumonia.

Alexander JE; Berry AM; Paton JC; Rubins JB; Andrew PW; Mitchell TJ

Department of Microbiology and Immunology, University of Leicester, Leicester, LE1 9HN, U.K.

Microb Pathog (ENGLAND) Mar 1998, 24 (3) p167-74, ISSN 0882-4010

Journal Code: MIC

Contract/Grant No.: AI34051, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Pneumolysin is a multi-functional toxin produced by *Streptococcus pneumoniae*. The toxin has distinct cytotoxic activity and complement-activating activity mediated by different parts of the toxin molecule. Mice challenged intranasally with a type 2 pneumococcal strain contract bronchopneumonia and bacteremia [1]. Mice were infected intranasally with isogenic mutants of this strain in which the chromosomal **pneumolysin** gene carried point **mutations** affecting either or both properties of **pneumolysin**. Reduction in either cytotoxic activity or complement activation by **pneumolysin** decreased the virulence of the **mutant** pneumococci. However, it was the ability to activate complement that most affected the behaviour of pneumococci in the lungs and associated bacteremia in the first 24 h following infection. Copyright 1998 Academic Press Limited.

2/3,AB/9

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09416003 98109732

A conserved tryptophan in pneumolysin is a determinant of the characteristics of channels formed by pneumolysin in cells and planar lipid bilayers.

Korchev YE; Bashford CL; Pederzolli C; Pasternak CA; Morgan PJ; Andrew PW; Mitchell TJ

Department of Cellular and Molecular Sciences, St. George's Hospital Medical School, London, U.K.

Biochem J (ENGLAND) Feb 1 1998, 329 (Pt 3) p571-7, ISSN 0264-6021

Journal Code: 9YO

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Pneumolysin is one of the family of thiol-activatable, cytolytic toxins. Within these toxins the amino acid sequence Trp-Glu-Trp-Trp is conserved. **Mutations** made in this region of **pneumolysin**, residues 433-436 inclusive, did not affect cell binding or the formation of toxin oligomers in the target cell membrane. However, the **mutations** did affect haemolysis, leakage of low-molecular-mass metabolites from Lettre cells and the induction of conductance channels across planar lipid bilayers. Of eight **modified** pneumolysins examined, Trp-433-->Phe showed the smallest amount of haemolysis or leakage (less than 5% of wild type). **Pneumolysin**-induced leakage from Lettre cells was sensitive to inhibition by bivalent cations but the extent of inhibition varied depending on the **modification**. Leakage by the **mutant** Trp-433-->Phe was least sensitive to cation inhibition. The ion-conducting channels formed across planar lipid bilayers exhibit small (less than 30 pS), medium (30 pS-1 nS) and large (more than 1 nS) conductance steps.

Small- and medium-sized channels were preferentially closed by bivalent cations. In contrast with wild-type toxin, which formed predominantly small channels, the modified toxin Trp-433-->Phe formed large channels that were insensitive to cation-induced closure. Polysaccharides of molecular mass more than 15 kDa inhibited haemolysis by wild-type toxin, but polysaccharide of up to 40 kDa did not prevent haemolysis by Trp-433-->Phe. Electron microscopy revealed that Trp-433-->Phe formed oligomeric arc and ring structures with dimensions identical with those of wild-type toxin, and that the ratio of arcs to rings formed was the same for wild-type toxin and the Trp-433-->Phe variant. We conclude that the change Trp-433-->Phe affects channel formation at a point subsequent to binding to the cell membrane and the formation of oligomers, and that the size of arc and ring structures revealed by electron microscopy does not reflect the functional state of the channels.

2/3,AB/10

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09388857 98114397

Role of tumor necrosis factor alpha in the host response of mice to bacteremia caused by pneumolysin-deficient *Streptococcus pneumoniae*.

Benton KA; VanCott JL; Briles DE

Department of Microbiology, The University of Alabama at Birmingham, 35294, USA.

Infect Immun (UNITED STATES) Feb 1998, 66 (2) p839-42, ISSN 0019-9567
Journal Code: GO7

Contract/Grant No.: AI21548, AI, NIAID; AI07051, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Pneumolysin-deficient **mutant** strains of *Streptococcus pneumoniae* are known to cause less-severe sepsis than wild-type pneumococcal strains that produce **pneumolysin**. This difference is associated with greater host resistance in mice infected with the **pneumolysin**-deficient strains. These studies show that the host resistance developed during the first 1 to 2 days after infection with a **pneumolysin**-deficient **mutant** strain is dependent on tumor necrosis factor alpha but is apparently independent of interleukin 1beta (IL-1beta) or IL-6. Survival beyond 5 days appeared to depend on the ability of the mice to produce IL-1beta.

2/3,AB/11

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09330050 98008785

The hemolytic and complement-activating properties of pneumolysin do not contribute individually to virulence in a pneumococcal bacteremia model.

Benton KA; Paton JC; Briles DE

Department of Microbiology, The University of Alabama at Birmingham, Birmingham, AL 35294-2170, USA.

Microb Pathog (ENGLAND) Oct 1997, 23 (4) p201-9, ISSN 0882-4010
Journal Code: MIC

Contract/Grant No.: AI21548, AI, NIAID; AI07051, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The virulence of pneumococcal capsular type 2 strain D39 and derivatives with **mutations** in the **pneumolysin** gene were examined in a mouse

bacteremia model. In CBA/N-XID mice D39 is known to exhibit exponential growth in the blood until the death of the mice at 24 to 36 h. In contrast, PLN, a pneumolysin-deficient derivative of D39, reaches a plateau in growth that is maintained for several days. The growth patterns of D39 and PLN observed in CBA/N-XID mice were also observed in C3H/HeJ and C3H/HeOuJ mice, but not in 129/SvJ and C57BL/6J mice. These results demonstrate that the effect of **pneumolysin** on bacteremia is dependent on the genetic background of the mice. D39 derivatives with point **mutations** which abolish the cytotoxic or complement-activating properties of **pneumolysin** did not have major individual effects on virulence in CBA/N- XID and C3H/HeOuJ mice. A derivative with **mutations** affecting both the cytotoxic and complement- activating properties resulted in a modest, yet statistically significant, increase in survival time of i.v. challenged CBA/N-XID mice. However, the effect was less marked than that seen with PLN. These findings suggest that the virulence effects of pneumolysin in bacteremia must be due in part to properties other than hemolysis and complement fixation. Copyright 1997 Academic Press Limited.

2/3,AB/12

DIALOG(R) File 155:MEDLINE(R)

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09227687 96197277

Distinct roles for pneumolysin's cytotoxic and complement activities in the pathogenesis of pneumococcal pneumonia.

Rubins JB; Charboneau D; Fasching C; Berry AM; Paton JC; Alexander JE; Andrew PW; Mitchell TJ; Janoff EN

Pulmonary Disease Division, Department of Medicine, Veterans Affairs Medical Center, Minneapolis, Minnesota, USA.

Am J Respir Crit Care Med (UNITED STATES) Apr 1996, 153 (4 Pt 1) p1339-46, ISSN 1073-449X Journal Code: BZS

Contract/Grant No.: AI31373, AI, NIAID; AI34051, AI, NIAID; DE-42600, DE, NIDR

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Pneumolysin, the major *Streptococcus pneumoniae* cytotoxin, contributes to the early pathogenesis of invasive pneumococcal pneumonia by facilitating intrapulmonary bacterial growth and invasion into the blood. Pneumolysin is a multifunctional toxin, with distinct cytolytic ("hemolytic") and complement-activation ("complement") activities that have been mapped to several regions of the molecule. To characterize the specific contributions of **pneumolysin** 's hemolytic and complement properties to the pathogenesis of pneumococcal pneumonia, we compared the in vivo effects of type 2 *S. pneumoniae* **mutant** strains, which produce pneumolysins deficient in these activities. The absence of either **pneumolysin**'s hemolytic or complement activities rendered **mutant** strains less virulent than the wild-type strain during pulmonary infection. **Pneumolysin** 's hemolytic activity correlated with acute lung injury and bacterial growth at 3 and 6 h after endotracheal instillation. In contrast, pneumolysin's complement activity correlated with bacterial growth and bacteremia at 24 h after pulmonary infection. **Pneumolysin** 's complement activity was not associated with the degree of alveolar-capillary injury or recruitment of leukocytes during initial pulmonary infection. However, **pneumolysin** 's complement activity inhibited killing of **mutant** bacteria in an in vitro complement-dependent neutrophil killing assay. Thus, both **pneumolysin** 's hemolytic and complement activities made specific contributions to the early pathogenesis of pneumococcal pneumonia at different stages of infection and by different mechanisms.

2/3,AB/13

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09075269 97229218

Streptococcus pneumoniae produces a second haemolysin that is distinct from pneumolysin.

Canvin JR; Paton JC; Boulnois GJ; Andrew PW; Mitchell TJ

Department of Microbiology and Immunology, University of Leicester, U.K.

Microb Pathog (ENGLAND) Mar 1997, 22 (3) p129-32, ISSN 0882-4010

Journal Code: MIC

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Pneumococci are described as alpha-haemolytic but under certain circumstances they produce zones of beta-haemolysis on blood-containing medium. This observation was investigated using wild type strains and a genetically-modified strain unable to produce the haemolytic toxin, **pneumolysin**. beta-haemolysis was produced by all pneumococci tested. It was not inhibited by anti-**pneumolysin** antibody but could be inactivated by cholesterol. These data confirm that pneumococci elaborate a second haemolysin, distinct from pneumolysin.

2/3,AB/14

DIALOG(R) File 155:MEDLINE(R)

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09020033 97230293

Differences in virulence for mice among *Streptococcus pneumoniae* strains of capsular types 2, 3, 4, 5, and 6 are not attributable to differences in pneumolysin production.

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Infect Immun (UNITED STATES) Apr 1997, 65 (4) p1237-44, ISSN 0019-9567 Journal Code: GO7

Contract/Grant No.: AI21548, AI, NIAID; AI07051, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

We observed that differences in the in vivo growth kinetics of pneumococcal strains of capsular types 3, 4, 5, and 6 were reminiscent of differences that we had previously reported for type 2 strain D39 and its **pneumolysin-deficient mutant**, PLN. Capsular type 2 *Streptococcus pneumoniae* D39 exhibits exponential growth in the blood of XID mice until the death of the mice at 24 to 36 h. In contrast, PLN reaches a plateau in growth that is maintained for several days. Capsular type 3 and 5 strains exhibited exponential growth and caused rapid death of XID mice following intravenous challenge, similar to the observation with D39. Strains of capsular types 4 and 6 exhibited growth kinetics reminiscent of PLN. Since the observed differences in the pathogenesis of types 3 and 5 compared to 4 and 6 were reminiscent of the effects of pneumolysin deficiency in type 2, we examined the levels of in vitro pneumolysin production for the entire panel of strains. The onset of pneumolysin production in most strains was rapid and occurred near the end of log-phase growth. Differences in in vivo growth patterns of capsular type 2, 3, 4, 5, and 6 strains were not found to be associated with differences in the levels of pneumolysin.

2/3,AB/15
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08459635 96082791
Pneumococcal virulence factors and host immune responses to them.
Watson DA; Musher DM; Verhoef J
Department of Veterinary and Microbiological Sciences, North Dakota State
University, Fargo 58105, USA.
Eur J Clin Microbiol Infect Dis (GERMANY) Jun 1995, 14 (6) p479-90,
ISSN 0934-9723 Journal Code: EM5
Languages: ENGLISH

Document type: JOURNAL ARTICLE; REVIEW; REVIEW, TUTORIAL
The principal virulence determinant of most encapsulated bacterial pathogens is the possession of an extracellular capsule. This paper discusses biological aspects of the *Streptococcus pneumoniae* capsule, putative roles played by accessory virulence factors of this pathogen and prospects for improvement of the currently available pneumococcal vaccine. Even though the interruption of genes encoding selected proteins has been shown to attenuate virulence to some degree, the physical removal of the pneumococcal capsule or the interruption of encapsulation genes completely abolishes virulence in mice. The role of the capsule in pathogenesis is not completely clear, however, since it is not known whether this structure is important in colonization, the obligatory first step in the process. In addition, a number of proteins have been implicated as possible accessory virulence factors. These include **pneumolysin**, two distinct neuraminidases, an IgA1 protease and two surface proteins, *pspA* and *psaA*. While interruption of the expression of some of these proteins examined to date has been shown to **attenuate** virulence, so far it has not proven possible to completely abolish virulence in this fashion. Proteinaceous accessory virulence factors may prove important to the development of second-generation pneumococcal vaccines, however. Pneumococcal and other proteins conjugated to pneumococcal polysaccharides are currently being evaluated as carriers in attempts to improve the immunogenicity of polysaccharide vaccines, primarily in small children.

2/3,AB/16
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08399622 95386973
The limited role of pneumolysin in the pathogenesis of pneumococcal meningitis.
Friedland IR; Paris MM; Hickey S; Shelton S; Olsen K; Paton JC; McCracken GH
Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, USA.
J Infect Dis (UNITED STATES) Sep 1995, 172 (3) p805-9, ISSN 0022-1899
Journal Code: IH3
Languages: ENGLISH

Document type: JOURNAL ARTICLE
The aim of this study was to determine the role of pneumolysin, an intracellular toxin of *Streptococcus pneumoniae*, in the pathogenesis of pneumococcal meningitis. Recombinant pneumolysin (1 microgram), when injected intracisternally into rabbits, resulted in a brisk inflammatory response. However, a pneumolysin-deficient strain of *S. pneumoniae* caused meningeal inflammation in rabbits indistinguishable from that induced by the parent **pneumolysin**-producing strain. Furthermore, similar

enhancement of meningeal inflammation occurred after ampicillin therapy in animals infected with either the parent strain or the **pneumolysin**-deficient **mutant**. These results suggest that although **pneumolysin** can stimulate the inflammatory cascade in the central nervous system, it is not necessary for the pathogenesis of meningeal inflammation nor does it play a role in postantibiotic enhancement of meningeal inflammation.

2/3,AB/17

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08359004 95330957

Growth and virulence of a complement-activation-negative mutant of *Streptococcus pneumoniae* in the rabbit cornea.

Johnson MK; Callegan MC; Engel LS; O'Callaghan RJ; Hill JM; Hobden JA; Boulnois GJ; Andrew PW; Mitchell TJ

Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, LA 70112, USA.

Curr Eye Res (ENGLAND) Apr 1995, 14 (4) p281-4, ISSN 0271-3683

Journal Code: DUB

Contract/Grant No.: EY00424, EY, NEI; EY02377, EY, NEI; EY08871, EY, NEI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Our previous work has demonstrated the importance of pneumolysin in the virulence of *S. pneumoniae* in a rabbit intracorneal model. This was accomplished by showing that deletion of the gene encoding pneumolysin resulted in reduced virulence, whereas restoration of the wild-type gene resulted in restoration of the virulent phenotype. To assess the importance of a particular domain in the **pneumolysin** molecule, we have now constructed a strain which produces a **pneumolysin** molecule which is hemolytic but which bears a site-specific **mutation** in the domain known to be associated with the complement-activating properties of this molecule. Comparison of the virulence of this strain with that of a strain bearing the wild-type gene showed statistically significantly lower total slit lamp examination (SLE) scores at 12, 18, 24, and 36 h (particularly with respect to fibrin formation), but no difference at 48 h. Determination of colony forming units (CFU) in eyes infected with the two strains showed approximately 10(6) bacteria per cornea until 36 h. Between 36 and 48 h, the bacteria were almost completely cleared with very few bacteria recoverable at the later time point. The loss of virulence observed with this **mutation** in the complement-activation domain of **pneumolysin**, though less than that observed with the gene deletion **mutant**, suggests that complement activation by **pneumolysin** has a significant role in the pathology observed in this model of corneal infection.

2/3,AB/18

DIALOG(R) File 155:MEDLINE(R)

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08302359 95247288

Effect of defined point **mutations** in the **pneumolysin** gene on the virulence of *Streptococcus pneumoniae*.

Berry AM; Alexander JE; Mitchell TJ; Andrew PW; Hansman D; Paton JC

Department of Microbiology, Women's and Children's Hospital, North Adelaide, South Australia.

Infect Immun (UNITED STATES) May 1995, 63 (5) p1969-74, ISSN

0019-9567 Journal Code: GO7

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The thiol-activated toxin **pneumolysin** is a known pneumococcal virulence factor, with both cytotoxic (hemolytic) and complement activation properties. Copies of the **pneumolysin** gene carrying defined point **mutations** affecting either or both of these properties were introduced into the chromosome of *Streptococcus pneumoniae* D39 by insertion-duplication mutagenesis. The virulences of these otherwise isogenic strains were then compared. There was no significant difference in either the median survival time or overall survival rate between mice challenged with D39 derivatives producing the wild-type toxin and those expressing a **pneumolysin** gene with an Asp-385-->Asn **mutation**, which abolishes the complement activation property. However, mice challenged with strains carrying either His-367-->Arg or Trp-433-->Phe plus Cys-428-->Gly mutations, which reduce hemolytic activity to approximately 0.02 and 0.0001% of the wild-type level, respectively, had significantly greater median survival times and overall survival rates than mice challenged with D39 derivatives expressing a wild-type pneumolysin gene. No additional reduction in virulence was observed when mice were challenged with a D39 derivative carrying Trp-433-->Phe, Cys-428-->Gly, and Asp-385-->Asn, rather than Trp-433-->Phe and Cys-428-->Gly, **mutations** in the **pneumolysin** gene. Thus, it appears that in the intraperitoneal challenge model, the contribution of **pneumolysin** to virulence is largely attributable to its hemolytic (cytotoxic) properties rather than to its capacity to activate complement. Interestingly, however, the amount of **pneumolysin** required for full virulence may be very small, as D39 derivatives carrying the Trp-433-->Phe **mutation** (which reduces hemolytic activity to 0.1% of the wild-type level) had intermediate virulence.

2/3,AB/19

DIALOG(R) File 155:MEDLINE(R)

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08258196 95122173

A **pneumolysin**-negative **mutant** of *Streptococcus pneumoniae* causes chronic bacteremia rather than acute sepsis in mice.

Benton KA; Everson MP; Briles DE

Department of Microbiology, University of Alabama at Birmingham.

Infect Immun (UNITED STATES) Feb 1995, 63 (2) p448-55, ISSN 0019-9567

Journal Code: GO7

Contract/Grant No.: AI21548, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Pneumolysin is a cytoplasmic virulence factor of *Streptococcus pneumoniae* that can interfere with phagocyte function in vitro. We have examined the effects of pneumolysin in vitro and in vivo and have found that it protects intravenously injected pneumococci against infection-induced host resistance. We employed a virulent capsular type 2 pneumococcal strain, D39, and its isogenic **pneumolysin**-negative **mutant**, PLN. Strain D39 exhibited exponential net growth in mice (doubling time, 1.4 h); 24 to 28 h after infection with 10(4) CFU, the numbers of pneumococci reached 10(9) to 10(10) CFU/ml and the mice died. Strain PLN yielded identical net growth in mice until reaching 10(6) to 10(7) CFU/ml at 12 to 18 h postinfection. At this time, the increase in the level of PLN CFU per milliliter ceased and remained constant for several days. PLN exhibited wild-type growth kinetics in mice when coinfecting simultaneously with strain D39. This observation suggests that pneumolysin exerts its effects

at a distance. By 12 to 18 h postinfection with PLN, mice exhibited the following evidence of an induced inflammatory response: (i) elevated plasma interleukin-6, (ii) a halt in the net growth of PLN, and (iii) control of the net growth of pneumolysin-producing D39 pneumococci upon subsequent challenge. Our data suggest that pneumolysin plays a critical role in sepsis during the first few hours after infection by enabling pneumococci to cause acute sepsis rather than a chronic bacteremia. However, once chronic bacteremia was established, it appeared that pneumolysin was no longer able to act as a virulence factor.

2/3,AB/20

DIALOG(R) File 155:MEDLINE(R)

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08256957 95114100

Dual function of pneumolysin in the early pathogenesis of murine pneumococcal pneumonia.

Rubins JB; Charboneau D; Paton JC; Mitchell TJ; Andrew PW; Janoff EN
Pulmonary Disease Division, Veterans Affairs Medical Center, Minneapolis, Minnesota.

J Clin Invest (UNITED STATES) Jan 1995, 95 (1) p142-50, ISSN 0021-9738 Journal Code: HS7

Contract/Grant No.: R29-AI34051, AI, NIAID; R29-AI31373, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Streptococcus pneumoniae is one of the most common etiologic agents of community-acquired pneumonia, particularly bacteremic pneumonia. Pneumolysin, a multifunctional cytotoxin, is a putative virulence factor for *S. pneumoniae*; however, a direct role for pneumolysin in the early pathogenesis of pneumococcal pneumonia has not been confirmed in vivo. We compared the growth of a pneumolysin-deficient (PLY[-]) type 2 *S. pneumoniae* strain with its isogenic wild-type strain (PLY[+]) after direct endotracheal instillation of bacteria into murine lungs. Compared with PLY(-) bacteria, infection with PLY(+) bacteria produced greater injury to the alveolar-capillary barrier, as assayed by albumin concentrations in alveolar lavage, and substantially greater numbers of PLY(+) bacteria were recovered in alveolar lavages and lung homogenates at 3 and 6 h after infection. The presence of pneumolysin also contributed to the development of bacteremia, which was detected at 3 h after intratracheal instillation of PLY(+) bacteria. The direct effects of pneumolysin on lung injury and on the ability of pneumococci to evade local lung defenses was confirmed by addition of purified recombinant pneumolysin to inocula of PLY(-) pneumococci, which promoted growth of PLY(-) bacteria in the lung to levels comparable to those seen with the PLY(+) strain. We further demonstrated the contributions of both the cytolytic and the complement-activating properties of **pneumolysin** on enhanced bacterial growth in murine lungs using genetically **modified pneumolysin** congeners and genetically complement-deficient mice. Thus, **pneumolysin** facilitates intraalveolar replication of pneumococci, penetration of bacteria from alveoli into the interstitium of the lung, and dissemination of pneumococci into the bloodstream during experimental pneumonia. Moreover, both the cytotoxic and the complement-activating activities of pneumolysin may contribute independently to the acute pulmonary injury and the high rates of bacteremia which characterize pneumococcal pneumonia.

2/3,AB/21

DIALOG(R) File 155:MEDLINE(R)

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08044950 95048813

Immunization of mice with pneumolysin toxoid confers a significant degree of protection against at least nine serotypes of *Streptococcus pneumoniae*.

Alexander JE; Lock RA; Peeters CC; Poolman JT; Andrew PW; Mitchell TJ; Hansman D; Paton JC

Department of Microbiology and Immunology, University of Leicester, United Kingdom.

Infect Immun (UNITED STATES) Dec 1994, 62 (12) p5683-8, ISSN 0019-9567 Journal Code: GO7

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Pneumolysin is the thiol-activated cytolysin produced by *Streptococcus pneumoniae*. Mice were immunized with a genetically engineered toxoid version of **pneumolysin**, which was derived from a serotype 2 pneumococcus. The toxoid carried the **mutation** Trp-433-->Phe. Alum was used as the adjuvant. Immunized mice had significantly increased levels of anti-**pneumolysin** antibodies, principally immunoglobulin G1. Mice were challenged intraperitoneally or intranasally with 12 strains covering capsular serotypes 1 to 6, 7F, 8, and 18C. Following challenge, the survival rate and/or the time of death of nonsurvivors (survival time) was significantly greater than that of sham-immunized mice for all nine serotypes. However, differences in the degree of protection were noted between different strains. The route of challenge also appeared to influence the degree of protection. Nevertheless, the significant, albeit in some cases partial, protection provided against all nine pneumococcal serotypes supports the conclusion that pneumolysin toxoids warrant consideration for inclusion in a human vaccine.

2/3,AB/22

DIALOG(R)File 155:MEDLINE(R)

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07980875 94345500

Characterisation of an oxidative response inhibitor produced by *Streptococcus pneumoniae*.

Perry FE; Elson CJ; Mitchell TJ; Andrew PW; Catterall JR

Department of Pathology and Microbiology, University of Bristol.

Thorax (ENGLAND) Jul 1994, 49 (7) p676-83, ISSN 0040-6376

Journal Code: VQW

Languages: ENGLISH

Document type: JOURNAL ARTICLE

BACKGROUND--Pneumonia caused by infection with *Streptococcus pneumoniae* is still a major clinical problem. Reactive oxygen species contribute to the killing of these bacteria by polymorphonuclear leucocytes (PMNs). Defence mechanisms of *Str pneumoniae* which counter reactive oxygen species are characterised. METHODS--PMNs were stimulated with phorbol myristate acetate (PMA) in the presence and absence of *Str pneumoniae* and supernatants from them, and superoxide (O₂⁻) production was measured by the reduction of ferricytochrome c. RESULTS--*Streptococcus pneumoniae*, but not *Klebsiella pneumoniae* or *Staphylococcus aureus*, inhibited PMA stimulated superoxide production by PMNs. Washed PMNs which had been preincubated with *Str pneumoniae* autolysis phase supernatants also exhibited depressed H₂O₂ production in response to PMA. The inhibitory activity was not attributable to non-specific cytotoxicity as assessed by release of the cytoplasmic enzyme lactate dehydrogenase, nor did the supernatants inhibit PMA stimulated degranulation of PMNs. Fractionation of the autolysis phase supernatants revealed inhibitory activity in both the fractions greater than and less than 10 kD. Like **pneumolysin** the inhibitory activity

was heat sensitive. However, both a parent and **pneumolysin** negative **mutant** Str pneumoniae, and autolysis phase supernatants from them, inhibited PMN superoxide production. Antisera to **pneumolysin** failed to abrogate the inhibitory effect of intact Str pneumoniae or autolysis phase supernatants from types 1 or 14 Str pneumoniae. CONCLUSIONS--The inhibitory effect of Str pneumoniae on the respiratory burst of PMNs is not shared by two other common lung pathogens. The existence of a novel inhibitor of the PMN respiratory burst, distinct from pneumolysin, has been demonstrated. The inhibitor is specific for the respiratory burst and is active both in the logarithmic phase of growth and during autolysis.

2/3,AB/23

DIALOG(R) File 155:MEDLINE(R)

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07843781 94011332

Identification of hydrogen peroxide as a Streptococcus pneumoniae toxin for rat alveolar epithelial cells.

Duane PG; Rubins JB; Weisel HR; Janoff EN

Department of Medicine, Minneapolis Veterans Affairs Medical Center, Minnesota.

Infect Immun (UNITED STATES) Oct 1993, 61 (10) p4392-7, ISSN 0019-9567 Journal Code: GO7

Contract/Grant No.: AI31373, AI, NIAID; R29-AI34051, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Streptococcus pneumoniae infections of the lung are associated with significant damage to the alveolar epithelium. Host phagocytes and pneumolysin, a cytolytic toxin of S. pneumoniae, are believed to contribute to this cellular damage, yet experiments in which these elements are absent demonstrate the presence of an additional soluble S. pneumoniae factor that is toxic to alveolar epithelium. We examined the effects of S. pneumoniae-associated alveolar epithelial cell injury by factors other than S. pneumoniae-derived **pneumolysin** or phagocyte products by exposing cultured rat type II alveolar epithelial cells (RAEC) to S. pneumoniae mutants that lacked **pneumolysin** activity. We found that **mutant pneumolysin**-deficient strains of S. pneumoniae produced injury to RAEC similar to that produced by the parent strains. A toxin of type 14 S. pneumoniae was distinguished from **pneumolysin** by physiochemical (i.e., molecular mass and heat stability) and functional (i.e., hemolytic activity and cytotoxic activity) properties and was identified as hydrogen peroxide. All S. pneumoniae strains tested produced hydrogen peroxide, and in many strains hydrogen peroxide production was comparable to that of activated neutrophils. We conclude that S. pneumoniae produces hydrogen peroxide in concentrations that are cytotoxic to RAEC in vitro and that alveolar epithelial damage due to hydrogen peroxide may be involved in the pathogenesis of host cellular injury in pneumococcal pneumonia.

2/3,AB/24

DIALOG(R) File 155:MEDLINE(R)

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07781835 94079341

Molecular analysis of the pathogenicity of Streptococcus pneumoniae: the role of pneumococcal proteins.

Paton JC; Andrew PW; Boulnois GJ; Mitchell TJ

Department of Microbiology, Adelaide Children's Hospital, Australia.

Annu Rev Microbiol (UNITED STATES) 1993, 47 p89-115, ISSN 0066-4227

Journal Code: 6DV

Languages: ENGLISH

Document type: JOURNAL ARTICLE; REVIEW; REVIEW, ACADEMIC

For many years the virulence of *Streptococcus pneumoniae* has largely been attributed to its antiphagocytic polysaccharide capsule. Recent evidence, however, indicates that certain pneumococcal proteins play an important part in the pathogenesis of disease, either as mediators of inflammation or by directly attacking host tissues. Pneumococci carrying defined **mutations** in the genes encoding any one of at least three pneumococcal proteins (the toxin **pneumolysin**, the major pneumococcal autolysin, and pneumococcal surface protein A) have significantly reduced virulence. Pneumococcal hydrolytic enzymes, such as neuraminidase, hyaluronidase, and IgA1 protease may also contribute to colonization and/or invasion of the host. Several of these proteins (or their detoxified derivatives) are protective immunogens in animal models and therefore warrant consideration for inclusion in human antipneumococcal vaccine formulations.

2/3,AB/25

DIALOG(R) File 155:MEDLINE(R)

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07558215 93275225

Immunization of mice with *Salmonella typhimurium* C5 aroA expressing a genetically toxoided derivative of the pneumococcal toxin pneumolysin.

Paton JC; Morona JK; Harrer S; Hansman D; Morona R

Department of Microbiology, Adelaide Children's Hospital, South Australia.

Microb Pathog (ENGLAND) Feb 1993, 14 (2) p95-102, ISSN 0882-4010

Journal Code: MIC

Languages: ENGLISH

Document type: JOURNAL ARTICLE

An **attenuated** *Salmonella* strain expressing a genetically toxoided derivative of the pneumococcal toxin **pneumolysin** was constructed by first transforming a methylation-positive, restriction-negative *Salmonella* with plasmid pJCP20M, a derivative of pBR322 containing the **modified pneumolysin** gene. Plasmid DNA was then extracted and transformed into *Salmonella typhimurium* C5 aroA. The transformant (denoted JM8) was capable of constitutively expressing the **modified pneumolysin** gene in vitro and stably maintained the recombinant plasmid containing the pneumococcal DNA, even in the absence of antibiotic selection. When JM8, or the parental *Salmonella* C5 aroA carrying pBR322 (denoted JM6), were administered orally to mice, both strains were capable of at least transient colonization of the Peyer's patches. Sera from JM8 mice (but not those fed JM6) had significant anti-pneumolysin IgG and IgA ELISA titres. Intraperitoneal administration of JM8 resulted in higher anti-pneumolysin IgG titres, but lower specific IgA levels.

2/3,AB/26

DIALOG(R) File 155:MEDLINE(R)

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07304329 92261297

Comparative efficacy of autolysin and pneumolysin as immunogens protecting mice against infection by *Streptococcus pneumoniae*.

Lock RA; Hansman D; Paton JC

Department of Microbiology, Adelaide Children's Hospital, North Adelaide, South Australia.

Microb Pathog (ENGLAND) Feb 1992, 12 (2) p137-43, ISSN 0882-4010
Journal Code: MIC

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Previous studies on *Streptococcus pneumoniae* have established that the pneumococcal proteins autolysin (N-acetylmuramyl-L-alanine amidase) and pneumolysin both contribute significantly to the virulence of the organism. In the present work, autolysin and a defined toxoid derivative of pneumolysin were tested, individually and in combination, for efficacy in a mouse model as antigens protecting against challenge with virulent, wild-type *S. pneumoniae*. While each antigen alone provided significant protection, the degree of protection was not increased when the antigens were administered together. In an additional experiment, mice were challenged with a genetically-modified mutant strain of pneumococcus unable to express active pneumolysin. Pre-immunization of such mice with autolysin failed to provide any significant protection against the challenge. The results of this study suggest that the most important contribution made by autolysin to the virulence of *S. pneumoniae* may be its role in mediating the release of pneumolysin from the pneumococcal cytoplasm during infection.

2/3,AB/27

DIALOG(R) File 155:MEDLINE(R)

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06926072 92030189

Pneumolysin induces the salient histologic features of pneumococcal infection in the rat lung in vivo.

Feldman C; Munro NC; Jeffery PK; Mitchell TJ; Andrew PW; Boulnois GJ; Guerreiro D; Rohde JA; Todd HC; Cole PJ; et al

Department of Thoracic Medicine, National Heart and Lung Institute, Brompton Hospital, London, United Kingdom.

Am J Respir Cell Mol Biol (UNITED STATES) Nov 1991, 5 (5) p416-23, ISSN 1044-1549 Journal Code: AOB

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Streptococcus pneumoniae infections are common, but how they cause host tissue injury and death is incompletely understood. Immunization with pneumolysin, a thiol-activated toxin produced by the pneumococcus, partially protects animals during subsequent infection. The mechanism by which pneumolysin contributes to disease is not known. The aim of the present investigation was to determine the histologic changes induced by recombinant pneumolysin in the rat lung and to compare them with the changes induced by live organisms. Injection of either toxin (200 or 800 ng) or bacteria into the apical lobe bronchus was associated with the development of a severe lobar pneumonia restricted to the apical lobe. The changes induced by the toxin were greater at the higher concentration, and changes were most severe in those animals in which there was partial ligation of the apical lobe bronchus. The pneumonitis was less severe following injection of a modified toxin with decreased hemolytic activity, generated by site-directed mutagenesis of the cloned pneumolysin gene, indicating that this property of the toxin was important in generating pulmonary inflammation. There was still considerable pneumonitis after injection of a modified toxin with decreased capacity to activate complement.

2/3,AB/28

DIALOG(R) File 155:MEDLINE(R)

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06866432 92114766

Complement activation and antibody binding by pneumolysin via a region of the toxin homologous to a human acute-phase protein.

Mitchell TJ; Andrew PW; Saunders FK; Smith AN; Boulnois GJ

Department of Microbiology, University of Leicester, UK.

Mol Microbiol (ENGLAND) Aug 1991, 5 (8) p1883-8, ISSN 0950-382X

Journal Code: MOM

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Pneumolysin, a membrane-damaging toxin, is known to activate the classical complement pathway. We have shown that 1 microgram ml⁻¹ of pneumolysin can activate complement, which is a much lower level than observed previously. We have identified two distinct regions of pneumolysin which show homology with a contiguous sequence within acute-phase proteins, including human C-reactive protein (CRP). Site-directed mutagenesis of the **pneumolysin** gene was used to change residues common to **pneumolysin** and CRP. Some of the **modified** toxins had a reduced ability both to activate complement and bind antibody. We suggest that the ability of **pneumolysin** to activate complement is related to its ability to bind the Fc portion of immunoglobulin G.

2/3,AB/29

DIALOG(R) File 155:MEDLINE(R)

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06579197 91249678

The role of pneumolysin in ocular infections with Streptococcus pneumoniae.

Johnson MK; Hobden JA; Hagenah M; O'Callaghan RJ; Hill JM; Chen S

Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, LA 70112.

Curr Eye Res (ENGLAND) Nov 1990, 9 (11) p1107-14, ISSN 0271-3683

Journal Code: DUB

Contract/Grant No.: EY00424, EY, NEI; EY08871, EY, NEI; EY02377, EY, NEI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Pneumolysin, a cytolytic protein produced by Streptococcus pneumoniae, has many properties which suggest it may be an important virulence factor in pneumococcal ocular infections. To directly test this possibility, we have constructed pneumolysin-negative strains of S. pneumoniae and compared their virulence with that of the wild type in a rabbit model of intracorneal infection. A **pneumolysin**-negative strain produced by chemical mutagenesis (probably a point **mutant**) was found to be no less virulent than the parent strain. However, a strain bearing a deletion in the **pneumolysin** gene showed greatly reduced virulence. This strain produced less pathology while showing significantly higher bacterial counts. These results suggest that a property of the pneumolysin molecule other than its cytolytic (hemolytic) activity may be involved in its pathogenic mechanism of action. This property may be the ability to activate complement, known to be a function of pneumolysin, which results in influx of PMNs, reducing the bacterial counts but also producing tissue damage.

2/3,AB/30

DIALOG(R) File 155:MEDLINE(R)

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06343401 88330217

The listeriolysin O gene: a chromosomal locus crucial for the virulence of *Listeria monocytogenes*.

Cossart P

Departement des Biotechnologies, Institut Pasteur, Paris.

Infection (GERMANY, WEST) 1988, 16 Suppl 2 pS157-9, ISSN 0300-8126

Journal Code: GO8

Languages: ENGLISH

Document type: JOURNAL ARTICLE

In culture supernatants of a Tn 1545-induced non-hemolytic mutant of *Listeria monocytogenes*, by immunoblotting with an anti-serum raised against purified listeriolysin O, we have detected the presence of a truncated protein of 52,000D (the secreted listeriolysin O is 60,000D). The region of insertion of the transposon has been cloned and sequenced. The transposon had inserted in an open reading frame. The homologies detected between this ORF, streptolysin O and **pneumolysin** demonstrate the the transposon had indeed inserted in the listeriolysin O gene. As the non-hemolytic **mutant** was non-virulent, our work demonstrated that a genetic determinant essential for virulence is the listeriolysin O gene or its adjacent region.

2/3,AB/31

DIALOG(R) File 155:MEDLINE(R)

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05579425 89307578

Pneumolysin, the thiol-activated toxin of *Streptococcus pneumoniae*, does not require a thiol group for in vitro activity.

Saunders FK; Mitchell TJ; Walker JA; Andrew PW; Boulnois GJ

Department of Microbiology, University of Leicester, United Kingdom.

Infect Immun (UNITED STATES) Aug 1989, 57 (8) p2547-52, ISSN 0019-9567 Journal Code: GO7

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The role of the single cysteine residue in the activity of the thiol-activated toxin **pneumolysin** was investigated using oligonucleotide-mediated, site-directed mutagenesis. Three **modified** toxins in which the cysteine residue was changed to an alanine, a serine, or a glycine residue were purified to homogeneity and examined for activity. The Cys-428----Ala modified toxin was indistinguishable from the wild-type recombinant toxin in terms of hemolytic activity and lytic and inhibitory effects on human polymorphonuclear leukocytes (PMN), indicating that the cysteine residue is not essential for toxin activity. The Cys-428----Ser and Cys-429----Gly modified toxins had reduced activity on erythrocytes and polymorphonuclear leukocytes, being 6 and 20 times less active than the wild type, respectively. However, all the modified toxins formed oligomers in erythrocyte membranes to the same extent as the wild-type recombinant toxin. This suggests that the cysteine residue at position 428 is involved in neither the binding of toxin to membranes nor its insertion into the membrane, and also that the formation of oligomers is not by itself sufficient for toxin activity.

2/3,AB/32

DIALOG(R) File 155:MEDLINE(R)

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05567646 89277519

Reduced virulence of a defined pneumolysin-negative mutant of Streptococcus pneumoniae.

Berry AM; Yother J; Briles DE; Hansman D; Paton JC

Department of Microbiology, Adelaide Children's Hospital, Australia.

Infect Immun (UNITED STATES) Jul 1989, 57 (7) p2037-42, ISSN 0019-9567 Journal Code: GO7

Contract/Grant No.: T32H007300; AI8557, AI, NIAID; AI21548, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Insertion-duplication mutagenesis was used to construct a pneumolysin-negative derivative of Streptococcus pneumoniae. This was achieved by first transforming the nonencapsulated strain Rx1 with a derivative of the vector pVA891 carrying a 690-base-pair DNA fragment from the middle of the pneumolysin structural gene. DNA was extracted from the resultant erythromycin-resistant, pneumolysin-negative rough pneumococcus and used to transform S. pneumoniae D39, a virulent type 2 strain. Several erythromycin-resistant transformants were obtained from two independent experiments, and none of these produced pneumolysin. Southern blot analysis confirmed that the pneumolysin gene in these transformants had been interrupted by the plasmid-derived sequences. The pneumolysin-negative mutants showed reduced virulence for mice compared with D39, as judged by survival time after intranasal challenge, intraperitoneal 50% lethal dose, and blood clearance studies. Pneumolysin production was reinstated in one of the mutants by transformation with the cloned pneumolysin gene, with the concomitant loss of erythromycin resistance; the virulence in mice of this isolate was indistinguishable from that of D39. These results confirm the involvement of pneumolysin in pneumococcal pathogenesis.

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